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Antimicrobial activity of per acetic acid for trans-operative disinfection of endodontic files

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Abstract— Reducing the accumulation of microorganisms on an endodontic file during endodontic treatment is important to limit recontamination of the root canal and increase likelihood of successful treatment outcome. Objective: To compare the antimicrobial activity of peracetic acid (PA), isopropyl alcohol and acetone against a range of bacteria and also for disinfection of contaminated endodontic K-files. Material and Methods: Antimicrobial activities of PA, isopropyl alcohol and acetone were compared against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, vancomycin resistant *E. faecalis* (VRE) and methicillin resistant *S. aureus* (MRSA), using minimum bactericidal concentration (MBC) and time-kill assays. Test solutions at different exposure times (15 s and 30 s) were assessed for treatment of endodontic files acting as carriers of *E. faecalis*-contaminated dental debris. Results: All bacteria were susceptible to PA (MBC range 0.25-1%), acetone (MBC range 50-60%) and isopropyl alcohol (30-40%). Using a time-kill assay of the antimicrobials at the determined MBC, all test microorganisms, with the exception of *E. faecalis* (VRE) 7766 were killed after 15 s exposure. In the case of *E. faecalis* 7766, viable cells remained detectable after 120 s

exposure to acetone. Testing disinfection of endodontic K-files, previously coated with dental debris containing *E. faecalis*, it was found that PA (2%) completely killed *E. faecalis* after 15 s exposure. However, even after 30 s exposure, isopropyl alcohol (80%) and acetone (80%) had limited disinfecting activity. Conclusion: Extrapolation of these results to clinical practice would suggest that PA would be the most effective agent for trans-operative disinfection of endodontic K-files during treatment of a single patient.

Keywords— Antimicrobial activity, biofilms, dental therapy, disinfectants, peracetic acid.

I. INTRODUCTION

It is well established that bacteria and their by-products are important factors in the development of pulp and periradicular pathogenesis[1]. Therefore, an essential component of endodontic treatment is the elimination of bacteria from the root canal system. This can be achieved by using chemical or mechanical procedures[2-4] Appliances used in endodontic treatment are either 'single use' or can be reused following sterilization by autoclaving (actual approaches used differ between countries)[5, 6]. As such, cross infection between patients

is not possible from the appliances used. However, during the single treatment of an individual patient, inadvertent reinfection of the root canal over this treatment may occur from the instrument being used⁷. During endodontic therapy, 2% chlorhexidine and 5% sodium hypochlorite (NaOCl) are frequently used irrigants to kill the microbiota present in the root canal system³. However, the cleansing and disinfection of instruments during single endodontic sessions, remains a matter of concern,^[5,6,8-10] as many of the approaches used are ineffective for removal of biological debris⁶. During single endodontic therapy, residual material can accumulate on the working surface of an endodontic instrument and maintenance of effective instrument disinfection during the treatment session is an important consideration^[8,9].

Peracetic acid (PA) is a recognized disinfectant that exhibits antibacterial, sporicidal, antifungal and antiviral properties. PA also lacks persistent toxic properties and does not generate mutagenic residuals or by-products^[10]. Having a broad-spectrum activity, even in the presence of heterogeneous organic matter, PA has previously been suggested for use as a sanitizing agent for gutta-percha cone disinfection^[11], for endodontic irrigation^[12], and also for smear layer removal in the root canal^[13].

The primary aim of this study was to evaluate and compare the antimicrobial activity of PA, isopropyl alcohol (isopropanol) and acetone (propanone) against a range of bacteria and also for the disinfection of contaminated endodontic K-files.

II. MATERIAL AND METHODS

Test solutions used were 2% peracetic acid (PA) (SekuseptTMAktiv - Henkel AG & Co. KGaA, Düsseldorf, DE), acetone (>97%) and isopropyl alcohol/isopropanol (>98%). Acetone and isopropyl alcohol were obtained from Merck (Darmstadt, DE).

The microorganisms tested included *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and vancomycin resistant *E. faecalis* (VRE) 7766, *E. faecalis* (VRE) 7767, methicillin resistant *S. aureus* (MRSA) 5963 and MRSA 6784. Non-ATCC strains originated from clinical samples.

The Minimal bactericidal concentration (MBC) of isopropyl alcohol, acetone and PA was initially determined. Briefly, standardized bacterial suspensions ($OD_{680nm}=0.1$; 107 to 108 cell/mL) were generated in Trypticase Soy Broth (TSB) and 5 μ L of these preparations transferred to sterile tubes. Aliquots (100 μ L) of isopropyl alcohol (concentration range 20% to 90% in water), acetone (concentration range 20% to 90% in water), or PA (concentration range 0.125% to 2% in

water) were then added to the bacterial suspensions for 120s. Sterile water was included in place of an antimicrobial as a negative control. A 100- μ L volume of double strength tryptone sodium chloride (TSC; 1g/L Tryptone (Difco), sodium chloride (Merck) 8.5g/L) was then added as a neutralizing agent, bacterial viability was then determined by plating 10 μ L portions of serial decimally diluted preparations in TSC on to an appropriate agar media and incubating for 24-48 h at 37°C.

The bacteria were cultured on Trypticase Soy Agar (TSA; Difco Laboratories) at 37°C for 24 h according to a method of the British Standards Institution (BS EN 1276)^[14]. Antimicrobial efficacy of isopropyl alcohol, acetone and PA (at the previously determined MBC) was measured after 15, 60 and 120 s exposure at room temperature (~25°C). Briefly, 1 mL of bacterial suspension (at an $OD_{660nm}=0.5$) was centrifuged in microtubes and the pellet resuspended in 1 mL of test disinfectant or sterile water (control). After appropriate contact time (15 s, 60 s or 120 s), 20 μ L aliquots of the suspension were transferred to 1.98 mL of TSC, which served as a neutralizing agent. After serial dilution in TSC, microbial suspensions were plated on to TSA and incubated at 37°C for 24 h. The number of CFU/mL was then determined for the test microorganisms.

The antimicrobial activity of isopropyl alcohol, acetone and peracetic acid on K-files contaminated with *E. faecalis* biofilms: Antimicrobial activities of test solutions (80% acetone, 80% isopropyl alcohol, and 2% PA) at different exposure times (15 and 30 s) were examined for endodontic files acting as carriers of *E. faecalis*-contaminated dental debris. Briefly, in vitro coronal access was performed to expose the root canal system of healthy teeth that had previously been extracted for orthodontic purpose (the study protocol was approved by the Institutional Ethics Committee from Rio de Janeiro State University – Brazil – Authorization 051-2009). The exposed tooth chamber was then placed inside a test tube containing 3 mL of TSB, and the preparation sterilized by autoclaving. *Enterococcus faecalis* ATCC 29212 ($OD_{660nm}=0.5$, 100- μ L) was inoculated into the test tubes to generate a biofilm on the dentinal surfaces^[15]. The preparation was incubated for 14 days with changes of TSB medium every 24 h to maintain biofilm growth. The teeth were then removed from the broths and the pulp chamber sealed with ColtosoITM (Coltene/WhaledentTM, New Jersey, USA). Teeth were immersed in a 5.25% NaOCl solution for 2 min to eliminate external contamination. The NaOCl was then neutralized using 10% sterile sodium thiosulphate solution for 2 min. The temporary coronal filling material was removed and K-endodontic files (#30K-type files, 21 mm long) were introduced into the contaminated root

canals with a filing motion until the flutes were visibly filled with dental debris. One group of K-files (n=30), with freshly removed biofilm (wet group) were immediately exposed to test antimicrobials in petri dishes (n=10 for each chemical test solution). For each chemical, the K-files were further divided into two groups of 5, being exposed to the agent at either 15 s or 30s. A second group (dehydrated) of K-files (n=30) were tested in the same manner, but these files were initially dried within a laminar flow chamber for 10 min prior to exposure to chemical agents. All files were transferred to test tubes containing Enterococcosel broth (BBL, Becton & Dickinson, Oxford, UK) and incubated for 48 h at 37°C, after which, bacterial growth was evident as a black precipitate within the medium. Controls were contaminated files exposed to saline and sterile files immediately immersed into test tubes containing Enterococcosel broth.

III. RESULTS

The MBCs for isopropyl alcohol, acetone and peracetic acid are presented in Table 1. Antimicrobial susceptibility to the chemical agents varied for the bacterial species. Enterococci were more resistant to acetone than other groups of microorganisms, with *P. aeruginosa* ATCC 27853 and MRSA 6784 found to be the most susceptible to isopropyl alcohol, and VRE 7767 and MRSA 5963 the most susceptible to PA. All strains were sensitive to PA concentrations $\geq 1\%$.

Table.1: Disinfectant activity evaluated by Minimal Bactericidal Concentration.

Microorganisms	TSC 120"	Acetone	Isopropanol	Peracetic acid
	Minimum Bactericidal Concentration			
<i>E. coli</i> ATCC 25922	2.5×10^6	50	40	1
<i>P. aeruginosa</i> ATCC 27853	2.6×10^6	50	30	1
<i>E. faecalis</i> ATCC 29212	1.6×10^6	60	40	1
<i>S. aureus</i> ATCC 25923	3.0×10^5	50	40	1
<i>S. aureus</i> MRSA 5963	1.6×10^6	50	40	0.25
<i>S. aureus</i> MRSA 6784	3.0×10^5	50	30	1
<i>E. faecalis</i> VRE 7766	3.5×10^5	60	40	1
<i>E. faecalis</i> VRE 7767	2.9×10^5	60	40	0.25

¹ Inoculum of viable bacteria in 5 µL incubated in TSC medium as negative control.

² Minimum bactericidal concentration of dilutions of disinfectant agents capable to kill all viable microorganisms ($\sim 10^6$ – 10^9 CFU) after contact to bacterial preparations (time kill) for 120 seconds. Isopropanol (Isopropyl alcohol) and acetone were used in concentrations varying from 90 to 20%, and Peracetic acid from 2 to 0.125%.

The antimicrobial activities of isopropyl alcohol, acetone and PA, were evaluated for several bacterial species at different contact times (Table 2). Contact with acetone, isopropyl alcohol, or 2% PA resulted in a total kill ($<3.3 \times 10^2$ CFU/ml; minimum level of detection of viable cells in this assay) after 15 s exposure. However, VRE 7766 remained detectable (2.3×10^4 CFU/mL) even after 120 s exposure to acetone.

Table.2: Disinfectant activity (time kill assay) after exposure to acetone (97%), isopropyl alcohol (98%) and peracetic acid (2%).

Microorganisms	*TSC	Acetone	Isopropanol	Peracetic Acid
<i>E. coli</i> ATCC 25922	9.0×10^9	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>P. aeruginosa</i> ATCC 27853	3.0×10^{10}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>E. faecalis</i> ATCC 29212	3.6×10^{10}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>S. aureus</i> ATCC 25923	4.5×10^{10}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>S. aureus</i> MRSA 5953	1.2×10^{12}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>S. aureus</i> MRSA 6784	1.2×10^{12}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>E. faecalis</i> VRE 7766	1.1×10^{11}	$^{**}2.3 \times 10^4$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$
<i>E. faecalis</i> VRE 7767	6.0×10^{10}	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$	$< 3.3 \times 10^2$

* Original inoculum of microorganisms exposed to 1 mL acetone (97%), isopropyl alcohol (98%) or Peracetic acid (2%), until 2 minutes.

Experiments using sessile *E. faecalis* grown on dentinal surfaces and transferred to the flutes of K-endodontic files were undertaken to establish the antimicrobial activity of the test chemicals under conditions closer to those encountered in the clinical environment. These studies showed that 15 s or 30 s exposure to both isopropyl alcohol and acetone (at 80%) did not eliminate bacterial contamination, especially when the files had previously been dried prior to exposure to the solutions (Table 3). In the case of contaminated and non-dried K-files exposed 15 s to 80% isopropyl alcohol, viable bacteria were not detected in 1 of 5 (20%) tested K-files. With longer exposure (30 s), 3 of 5 K-files (60%) were found to be free of contamination. Antimicrobial effects were also evident for freshly contaminated files following exposure to acetone for both test periods (Table 2). In these experiments, exposure to isopropyl alcohol for 30 s was more effective than for acetone ($p < 0.05$, chi-square test). Experiments performed with concentrated isopropyl alcohol or acetone failed to completely eliminate bacterial biofilms after 30 s exposure times. PA demonstrated the greatest antimicrobial activity being able to eliminate viability of both freshly and dried sessile *E. faecalis* cells on all K-endodontic files after exposure for 15 s (100%).

Table.3. Antimicrobial activity of Isopropyl Alcohol (80%), Acetone (80%) and Peracetic acid (2%) on K-files (carrier test) contaminated with *E. faecalis* grown in biofilms on dentin matrix (percentage of elimination).

Chemical	Period of exposure	Wet ¹	Dehydrated ¹
Isopropyl alcohol	15 ^{''}	20%	0%
	30 ^{''}	60%	20%
Acetone	15 ^{''}	20%	0%
	30 ^{''}	20%	0%
Peracetic acid	15 ^{''}	100%	100%
	30 ^{''}	100%	100%

¹ K-files used as carriers were maintained inside a sterile humidified chamber (wet) or dried inside laminar flow chamber previously to the exposure to chemicals for 15 or 30 seconds.

IV. DISCUSSION

Microorganisms can remain viable on the surfaces of endodontic instruments for varying lengths of time and as such they may act as reservoirs of re-contaminating organisms during a single treatment period[16].

Concern over reducing microbial load on the surfaces of dental instruments during the same treatment period has arisen in recent years. This is largely due to previous cross-contamination of individuals through non-autoclavable instruments such as bronchoscopes, endoscopes [17] and other apparatus like dental chairs waterlines[18,19].

Of additional concern has been the potential acquisition of microbial resistance[20] to frequently used disinfectants and antiseptic agents[21]. Therefore, continuous monitoring of resistance profiles to disinfectant agents is highly important to ensure safety within clinical practice.

Although stated as disinfectants[22], to our knowledge, relatively few reports have tested the sensitivity of bacteria to both isopropyl alcohol and acetone, motivating inclusion of these agents in comparable tests with PA.

The approaches used to clean endodontic instruments are generally ineffective for the removal of biological debris, and therefore, single use instruments are advocated to avoid cross-infection occurring between patients. However, for a single patient, the same endodontic file may be used throughout the treatment process and it is possible that this instrument may, in effect, 're-infect' the root canal during the treatment. Since one of the key objectives of endodontic therapy is eradication of bacteria, effective disinfection of instruments during such a single treatment procedure would be beneficial[16,22,23].

In the present study, differences in antimicrobial susceptibility of microorganisms were detected in relation

to microbial growth pattern (sessile or planktonic). Bacteria growing as biofilms have distinctive features compared with the same bacteria growing planktonically (free floating). For example, biofilm cells are frequently more tolerant to antimicrobial agents, conditions of stress and host defenses, compared with their planktonic equivalents. This recalcitrance of biofilms makes them extremely difficult to treat[23].

In our results, microorganisms grown planktonically were highly susceptible to all disinfectant agents after evaluation by a time-kill assay. A reduction in bacterial viability higher than 105 CFU/ml was observed for all test solutions, indicating that the chemicals had adequate disinfecting activity for planktonically grown Gram-negative and Gram-positive bacteria after exposure for 15 s. The only exception was VRE 7766 which, by time kill assay, demonstrated a residual number of viable cells (2.3×10^4 CFU/mL) after exposure of $\sim 10^{11}$ CFU/ml to concentrated acetone[16,17,22,23].

Determination of the MBC for all microbial strains also reinforced the susceptibility of planktonic microorganisms. The MBC, determined by exposure for 2 min to different concentrations of the tested chemical agents, demonstrated that all strains were sensitive to PA concentrations $\geq 1\%$, and isopropyl alcohol was more active than acetone in reducing viability of bacteria ($\sim 10^5$ - 10^6 CFU), including the VRE 7766. Therefore, as isopropyl alcohol had a lower MBC concentration (40%) than acetone (60%), it should theoretically be more effective in clinical use[23,24].

Whilst isopropyl alcohol and acetone are ineffective at killing bacterial spores, maintenance of decontamination of instruments by vegetative organisms should be achievable by these agents[24]. Importantly, microorganisms exhibiting resistance to antibiotics or antimicrobial agents often also have higher tolerance to disinfectants[25]. This study therefore also evaluated activity of acetone, isopropyl alcohol and PA to both MRSA and VRE strains, and the test agents were shown to inhibit the viability of planktonic forms of these microorganisms. In contrast, the effectiveness of disinfectants against microorganisms grown in biofilms was relatively limited. In this study, sessile *E. faecalis* removed from dentine walls by K-files used in the carrier test, exhibited resistance to acetone and isopropyl alcohol after 30 s exposure. This was particularly evident when contaminated K-files were dried prior to exposure to the disinfectant, even at higher concentrations. The volatility of both isopropyl alcohol and acetone might lead to variations in their concentrations within clinical situations[26,27]. As a result, our experiments using K-files in carrier tests were conducted with acetone and isopropyl alcohol diluted to 80%. This finding suggests that biofilm growth in dentine matrices is an important

factor for microbial resistance to acetone or isopropyl alcohol. Furthermore, in skin surface experiments, microorganisms are usually sensitive to alcohol-based antiseptics, with studies showing that the most effective antimicrobial activity occurs with 2% chlorhexidine digluconate in 70% isopropyl alcohol[26]. Moreover, isopropyl alcohol-based hand antiseptics have demonstrated higher activity against *E. coli*, *Micrococcus luteus*, and *S. aureus* than ethanol-based disinfectants in experiments on skin surfaces[27].

Overall, 2% PA was deemed to be the best disinfectant, as it was able to eliminate all microbial isolates, regardless of their growth form, or test method used. Significantly, total elimination of microbial viability occurred with sessile cells derived from dentinal matrices for all K-file carriers (100%) after 15 s exposure[25-27]. PA is an oxidizing agent used in the decontamination of a wide range of medical equipment as well as in food and water treatment processes[10]. PA disinfection is rapid and effective against bacteria, fungi, viruses and spores[10-12, 28-30].

In this study, a modified K-file carrier test was developed that enabled the assessment of disinfectant efficacy against biofilm microorganisms in the presence of dentine debris. The method was relatively easy to perform, and simulated conditions observed in clinical practice, in which the files may present a dense biofilm contamination.

V. CONCLUSION

Based on the study findings, it was concluded that PA was the most effective of the test disinfectants and as such is advocated as an appropriate disinfectant for single use endodontic instruments during use in a single treatment session. We suggest the use of 2% PA inside the 'endo stand', to allow all instruments being used in the canal treatment be kept at hand in the order of their use and disinfection.

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